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Short Communication

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## The Role of Mealybug-Induced Cassava Plant Volatiles in the Attraction of the Encyrtid Parasitoids *Aenasius vexans* and *Apoanagyrus diversicornis*

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### INTRODUCTION

*Aenasius vexans* Kerrich and *Apoanagyrus (Epidinocarsis) diversicornis* Howard (Hymenoptera: Encyrtidae) are larval parasitoids of the cassava mealybug, *Phenacoccus herreni* Williams & Cox (Sternorrhyncha: Pseudococcidae). To control epidemic outbreaks of this cassava pest in South America, they were used in successful mass release programs (Bellotti *et al.*, 1999). It was shown in a previous study (Bertschy *et al.*, 1997) that both encyrtid wasps are attracted to infested cassava plant odors and preferred these significantly over healthy plant odors. As yet, it is unclear whether this increased attractiveness is due to substances emanating from the mealybugs and/or their by-products or if it is the result of changes in volatile emissions by the plant due to mealybug infestation.

Herbivore hosts can be located by parasitoids by using plant-provided cues, as has been shown, e.g., for lepidopteran herbivores (Mattiacci *et al.*, 1994; Turlings *et al.*, 1995). It has been argued that cues originating directly

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from the host may play a less important role in the foraging behavior of parasitoids due to their minute quantities (Vet and Dicke, 1992). In the case of mealybugs and related insects, however, large amounts of by-products are deposited in the vicinity of the host. Among the by-products, honeydew has received much interest for its possible importance for parasitoids and predators (van Vianen, 1989). Chemical substances in honeydew and other sternorrhynchan by-products have been found mostly to be arrestants for parasitoids or to stimulate close-range searching behavior (e.g., Bouchard and Cloutier, 1984; van Roermund and van Lenteren, 1995). In some cases, they also elicit long-range attraction in parasitoids of whiteflies and aphids (e.g., Guerrieri, 1997; Bouchard and Cloutier, 1985; Wickremasinghe and van Emden, 1992).

The attractivity of mealybugs for parasitoids has been tested in a system using cassava, its African mealybug pest *Phenacoccus manihoti*, and the parasitoid *Apoanagyrus lopezi*, yielding variable results. While infested cassava plants were shown to be attractive to the parasitoid, healthy plants did not attract, nor did mealybugs with their odor by-products (Nadel and van Alphen, 1987). In contrast, van Baaren and Nénon (1996) found, for the same system, that unparasitized mealybugs attracted the wasps. Soussi *et al.* (1998) confirmed that unparasitized mealybug odors were attractive to *A. lopezi* and demonstrated that these cues were even preferred over the odors from healthy cassava plants. The wasp also distinguished plants that carried unparasitized versus parasitized mealybugs.

For the system used in our study involving the Latin American mealybug species *P. herreni*, the source of attraction of *A. vexans* and *A. diversicornis* to infested cassava plants remains to be elucidated (Bertschy *et al.*, 1997). The ample presence of mealybugs and their by-products could play a role, considering the development and behavior of *P. herreni* (Bellotti *et al.*, 1984). The larvae remain within the ovisac for a short time after hatching and then migrate to the apical part of the young growing shoots of cassava plant. When the mealybug population increases, they will disperse to all plant parts. After they find a feeding site they mostly remain there if not disturbed. Because *P. herreni* females spend their whole lifetime on the same plant, they are always surrounded by their excretion products such as exuviae, feces, defense substances, honeydew, and the sooty mold that forms on it. On the other hand, plant volatiles induced by *P. herreni* could also play an important role, particularly since *A. diversicornis* showed a preference for infested over healthy cassava plants (Bertschy *et al.*, 1997). In the current study, responses of *A. vexans* and *A. diversicornis* to various odor sources in an olfactometer were quantified to determine the attractiveness of plant-derived volatiles as opposed to mealybug-derived volatiles.

## MATERIALS AND METHODS

Plants and insects were reared as described by Bertschy *et al.* (1997). Three weeks after planting, experimental cassava plants (*Manihot esculenta* Krantz, var. CMC40) were infested with mealybug ovisacs as described by van Driesche *et al.* (1987). Approximately 6 weeks after planting, healthy and infested plants were used in experiments. The parasitoids *A. vexans* and *A. diversicornis* were reared from mealybugs on cassava plants in cages, and they were used 1.5 to 2.5 days after emergence, at which age they respond well to odors (Bertschy *et al.*, 1997). The wasps were offered a choice between odors in a Y-tube olfactometer similar to the one designed by Sabelis and van de Baan (1983), following the procedure described by Bertschy *et al.* (1997). In principle, odor sources were placed in tubular glass chambers of 6.5-cm diameter. The chambers were connected to the two arms of the olfactometer. Activated charcoal-filtered air entered each arm of the olfactometer at a flow rate of 400 ml/min. Prior to each test, female parasitoids were given an oviposition or ovipositor probing experience on a mealybug-infested cassava leaf. The wasps were given this experience to increase their responsiveness, which is usually poor for inexperienced parasitoids (Turlings *et al.*, 1993; Vet *et al.*, 1995). After experience, the wasps were released individually from gelatine capsules at the entrance of the olfactometer. The position of the parasitoid was recorded after 5 min and, again, after 10 min. Behavior was classified as no-choice if the parasitoid remained in the common arm of the olfactometer or passed less than 8 cm in an arm and as choosing one of the odor sources if it had passed more than the first 8 cm of one of the arms (Bertschy *et al.* 1997). To initiate movement of the insect, a spotlight (100 W) was placed in front of the Y-tube behind a white curtain fixed on a wooden frame which was placed around the olfactometer. For statistical analyses, a chi-square test was applied, using the total number of females that made a choice for a particular odor ( $\alpha = 0.05$ ).

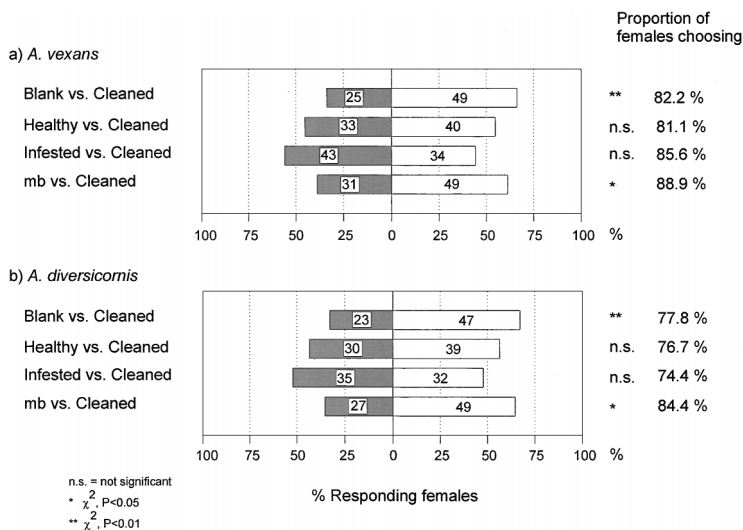
To determine if the attractiveness of infested plants was due to odors emitted by the plant or to odors from mealybugs and their by-products, these two potential sources were tested separately and in combination. For this purpose, cassava leaves infested with mealybugs and carrying exuviae, as well as honeydew and sooty mold, were carefully cleaned. First, the mealybugs were removed with a fine paintbrush and then all the products left on the leaves were carefully wiped off with wet cotton wool and distilled water, without wounding the leaves. These leaves, free of their pests and by-products, were assumed to release only plant-produced odors. The petiole of each washed leaf ("cleaned") was wrapped in wet sterilized cotton wool to avoid any odor emanating from the cut petiole. Two such leaves were then

placed in the olfactometer and tested against other potential odor sources. Sterilized cotton wool was used because normal cotton wool had been found to be somewhat attractive to the wasps (Bertschy, unpublished data). The removed mealybugs and by-products ("mealybugs") were placed on pieces of wet cotton wool of the same size and used as an odor source. "Cleaned" and "mealybugs" were tested against each other and against (1) two infested leaves ("infested"), which carried mealybugs as well as honeydew and sooty mold, but no other arthropod species; (2) two healthy leaves ("healthy"), which were absolutely free of arthropod pests and had never carried any; and (3) a control ("blank"), consisting of only sterilized wet cotton wool. A combination of healthy leaves and mealybugs and their by-products ("healthy + mealybugs") was also tested against infested leaves to confirm the presence of induced volatiles.

## RESULTS AND DISCUSSION

This study confirms that odors may play an important role for the parasitoids *Aenasius vexans* and *Apoanagyrus diversicornis* in the location of plants that are infested by their principle host, the cassava mealybug. Previously we found that the odor of mealybug-infested cassava plants was highly attractive to these wasps (Bertschy *et al.*, 1997), but in that first study, the tendency of *A. vexans* to chose the odor of healthy cassava plants over clean air ("blank") was not statistically significant. Here we repeated this experiment, increasing the number of parasitoid wasps from 62 to 90. This time there was a statistically significant preference for healthy leaf odors, with 67.2% of females choosing this odor source ( $\chi^2 = 6.89$ ,  $P < 0.01$ ). Thus, both parasitoid species, *A. vexans* and *A. diversicornis*, showed similar responses; they are attracted to the odor of cassava plants, but when given a choice they prefer the odor of *P. herreni*-infested cassava over the odor of healthy cassava (Bertschy *et al.*, 1997).

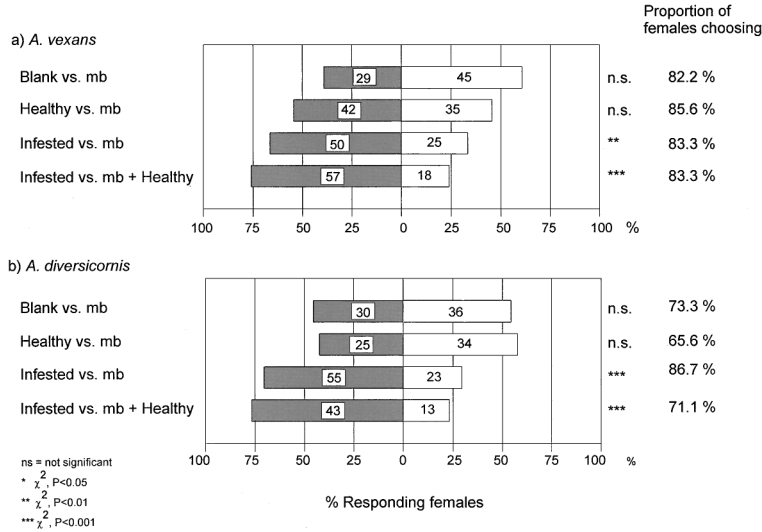
The current study shows that attractive odors are mainly of plant origin and not emitted by materials that are more directly associated with mealybugs. The odor of cleaned infested leaves was preferred by both species over clean air ( $\chi^2 = 7.78$ ,  $P < 0.01$ , and  $\chi^2 = 8.22$ ,  $P < 0.005$ , respectively, for *A. vexans* and *A. diversicornis*), as well as over the odor of mealybugs and their by-products ( $\chi^2 = 4.05$ ,  $P < 0.05$ , and  $\chi^2 = 6.36$ ,  $P < 0.025$ ) (Fig. 1). Moreover, neither wasp species distinguished "cleaned" leaves from fully infested ones ( $\chi^2 = 1.05$ ,  $P > 0.25$ , and  $\chi^2 = 0.134$ ,  $P > 0.50$ ), while we know that they do distinguish infested from healthy leaves (Bertschy *et al.*, 1997). Somewhat confusing is the fact that they did not readily distinguish between "cleaned" leaves and healthy leaves ( $\chi^2 = 0.67$ ,  $P > 0.25$ , and



**Fig. 1.** Responses of (a) *A. vexans* and (b) *A. diversicornis* in a Y-tube olfactometer to the odors of infested cassava plants from which mealybugs and by-products had been removed (cleaned) in a choice next to clean air (blank), healthy uninfested cassava leaves (healthy), fully infested cassava leaves (infested), or mealybug plus by-products (mb). In each bar the actual number of wasps that made a particular choice is given, while the X axis indicates the percentages of choosing wasps that these numbers represent. Next to the bars the proportion of females that made a choice for one of the two odors is given ( $n = 90$  for each combination).

$\chi^2 = 1.17$ ,  $P > 0.25$ ). This may indicate that the cleaned cassava leaves released less of attractive substances than fully infested leaves, perhaps because of the absence of actively feeding insects and the removal of attractive substances while washing the leaves. Active feeding by insects may increase plant odor emissions and attractiveness to parasitoids as found by Turlings *et al.* (1991).

The mealybugs plus associated by-products failed to attract either parasitoid species in the absence of plant odor (Fig. 2). When they were given a choice between this odor source and clean air, 60.8% of the *A. vexans* females walked up to the arm leading to the mealybugs, which is just below the level of significance ( $\chi^2 = 3.459$ ,  $0.1 < P > 0.05$ ). In the case of *A. diversicornis*, only 54.5% of the responding females chose this odor over pure air ( $\chi^2 = 0.545$ ,  $P > 0.5$ ). Surprisingly, the wasps did not distinguish between healthy leaves and mealybugs plus by-products, although healthy leaves have been found to be attractive to both encyrtids (Bertschy *et al.*, 1997). Perhaps, along with the removal of the mealybugs and by-products, some plant substances



**Fig. 2.** Responses of (a) *A. vexans* and (b) *A. diversicornis* in a Y-tube olfactometer to the odor of mealybugs and their by-products (mb) in a choice next to clean air (blank), healthy uninfested cassava leaves (healthy), or fully infested cassava leaves (infested). In the last combination the mealybugs were combined with healthy leaves and tested against infested leaves. In each bar the actual number of wasps that made a particular choice is given, while the  $X$  axis indicates the percentages of choosing wasps that the numbers represent. Next to the bars the proportion of females that made a choice for one of the two odors is given ( $n = 90$  for each combination).

were removed and added to the mealybug odor, thus rendering them slightly attractive. Indications for similar effects were reported by Boevé *et al.* (1996) in a quantitative study where they removed the herbivor's by-products from the infested plant. Minute amounts of a behaviorally active terpene found in these by-products were considered a contamination from the plant surface, on which the terpene occurred in large quantities. Further, Read *et al.* (1970) showed in a tritrophic system that aphids removed from their food plants were initially attractive to their parasitoid, the braconid *Diaretiella rapae*, but not 24 h after removal, which also indicates similar effects.

Odors from infested leaves were significantly more attractive to both *A. vexans* and *A. diversicornis* than mealybug odors ( $\chi^2 = 8.33$ ,  $P < 0.005$ , and  $\chi^2 = 13.12$ ,  $P < 0.001$ , respectively). This confirms that odors of plant origin are dominant in the attractiveness and are more important than odors of direct mealybug origin. Moreover, infested leaves were clearly preferred over the combination of healthy leaves and a removed mealybug complex ("healthy + mealybugs") ( $\chi^2 = 20.28$ ,  $P \ll 0.001$ , and  $\chi^2 = 16.07$ ,  $P \ll$

0.001, respectively, for *A. vexans* and *A. diversicornis*). The combined results strongly suggest that the increased attractiveness of infested leaves is caused by herbivore-induced odor emissions.

The wasps' choices were not unambiguous, which indicates that each of the treatments tested was somewhat attractive, but it may also be caused by variability in preferences or motivation among females. It should be emphasized that we used wasps that experienced all components of the plant–host complex prior to release in the olfactometer, which is likely to have increased their subsequent responsiveness to the odors of these components (Turlings *et al.*, 1993; Vet *et al.*, 1995). Since the wasps will experience the same in the field and since all potentially important volatile substances were encountered during these experiences, the observed responses can be expected to reflect those of females in their natural environment.

The lack of parasitoid attraction to mealybugs and their by-products in our South American systems contrasts with certain recent findings in the African system. Both van Baaren and Nénon (1994), and Souissi *et al.* (1998) and Souissi and Le Rü (1999) reported a positive response of the female parasitoid *A. lopezi* to the mealybug *P. manihoti*, using different experimental procedures. This response was found shortly after mealybug removal from the plant, but no longer at an interval of 24 h (Souissi and Le Rü, 1999). In our system, the mealybugs elicited no attraction despite the short interval of 0.5 to 3 h between removal and testing. The clear presence of mealybug-induced volatiles coincides well, on the other hand, in both tritrophic systems.

Induced volatiles released by infested plants are, unlike host-derived volatiles, usually released in large quantities and relatively reliable cues for host presence (Vet and Dicke, 1992). As is true for other parasitoids of herbivores, *A. vexans* and *A. diversicornis* seem to rely predominantly on cues emitted by host-infested plants. Once the wasps are on an infested plant, they may use mealybug-derived stimuli to confirm the presence of hosts and to pinpoint their exact location on the plant. Contact kairomones and relatively low concentrations of odors released by the host and its by-products should be sufficient for this short-range searching behavior.

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